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SUMMARY

DNA damage triggers chromatin remodeling by mechanisms that are poorly understood. The oncogene and chromatin remodeler ALC1/CHD1L massively decompacts chromatin *in vivo*, yet is inactive prior to DNA-damage-mediated PARP1 induction. We show that the interaction of the ALC1 macrodomain with the ATPase module mediates auto-inhibition. PARP1 activation suppresses this inhibitory interaction. Crucially, release from auto-inhibition requires a poly-ADP-ribose (PAR) binding macrodomain. We identify tri-ADP-ribose as a potent PAR-mimic and synthetic allosteric effector that abrogates ATPase–macrodomain interactions, promotes an ungated conformation and activates the remodeler's DNA-dependent ATPase. ALC1 fragments lacking the regulatory macrodomain relax chromatin *in vivo* without requiring PARP1 activation. Further, the ATPase restricts the macrodomain's interaction with PARP1 unless DNA damage is induced. Somatic cancer mutants disrupt ALC1's auto-inhibition and activate chromatin remodeling. Our data show that the NAD^+-metabolite PAR triggers ALC1 to drive chromatin relaxation. Thus, modular allostery in this oncogene tightly controls its robust, DNA-damage-dependent activation.

INTRODUCTION

Chromatin structure safeguards the integrity of our genome. Distinct families of chromatin remodeling enzymes establish and maintain chromatin structure, for example by facilitating the binding of transcription factors to functional DNA elements or assisting the repair machinery upon DNA damage. Key to controlling the activity of these ATPases are chromatin targeting mechanisms and regulatory interactions. Such mechanisms help ensure that remodelers are only active where and when needed. While the mechanisms through which the Chd1 and ISWI remodelers are regulated by nucleosomes have been explored (Clapier and Cairns, 2012; Hauk et al., 2010; Ludwigsen et al., 2017; Yan et al., 2016), less is known about how DNA damage triggers the activity of remodelers such as the PARP1-dependent ALC1 (Ahel et al., 2009; Gottschalk et al., 2012; 2009), which massively decompacts chromatin upon DNA damage [**Movie S1** and (Sellou et al., 2016)]. Considering *ALC1*'s validated roles as an oncogene (*ALC1* is amplified in several cancers, promotes metastases, proliferation and pluripotency (Chen et al., 2010; Jiang et al., 2015; Kulkarni et al., 2013; Ma et al., 2007)), understanding how PAR triggers ALC1 activity would advance our molecular understanding of how DNA damage impacts our genome, shed light on how a NAD^+ metabolite and nucleic acid triggers the activation of an oncogene, and reveal approaches that might allow us to target ALC1 therapeutically.

Single-strand DNA breaks rapidly induce the activity of PARP1, PARP2 and PARP3, enzymes that use NAD^+ to ADP-ribosylate chromatin and other cellular targets (Carter-O'Connell et al., 2016; Gibson et al., 2016; Grundy et al., 2016). The clinical promise of PARP1 inhibitors in cancer therapy (Lord and Ashworth, 2017) and the identification of domains that recognize ADP-ribosylated proteins, including ADP-ribose binding macrodomains (Karras et al., 2005; Kustatscher et al., 2005), has rekindled interest in NAD signaling (Cambronne et al., 2016; Kim et al., 2004; Petesch and Lis, 2012; Tulin and Spradling, 2003). While cellular mono-

ADP-ribosylation, catalyzed by related PARP enzymes, is thought to act as a reversible, regulatory post-translational modification (PTM) (Jankevicius et al., 2013; Rosenthal et al., 2013; Sharifi et al., 2013), the tightly regulated synthesis of PAR by PARP1 and PARP2 profoundly alters nuclear organization and cellular homeostasis (Altmeyer et al., 2015; Asher et al., 2010; Bai et al., 2011a; 2011b; Wright et al., 2016).

PAR is a nucleic acid with important roles in the stress response to DNA damage. It is as an abundant, transient polymeric anion that can promote phase separation (Altmeyer et al., 2015; Asher et al., 2010; Bai et al., 2011b; 2011a; Wright et al., 2016). Sites of high PARP1 activity *in vivo* recruit ATP-dependent remodelers, including ALC1 (Amplified in Liver Cancer 1; also known as CHD1L), CHD2, CHD4, SMARCA5/SNF2H and *Drosophila* Mi-2 (Chou et al., 2010; Murawska et al., 2011; Polo et al., 2010; Smeenk et al., 2013). Remodelers such as ALC1 and CHD2 mediate chromatin relaxation through unknown mechanisms at the site of DNA damage (Luijsterburg et al., 2016; Sellou et al., 2016). These are some of the earliest, PARP-dependent changes in chromatin structure upon DNA damage (Kruhlak et al., 2006; Poirier et al., 1982; Strickfaden et al., 2016).

We and others have shown that ALC1 recruits to DNA damage sites upon PARP1 activation. Recruitment requires its C-terminal, PAR-binding macrodomain module (Ahel et al., 2009; Gottschalk et al., 2012; 2009). Interestingly, its ATPase and nucleosome-remodeling activities depend on PARP1 activitation. *In vitro* assays reveal that PARylated PARP1 promotes ALC1-dependent nucleosome sliding (Ahel et al., 2009; Gottschalk et al., 2012; 2009). Key to ALC1's activity is the ability of its macrodomain to recognize PARylated PARP1. However, what keeps ALC1 inactive prior to PARP1 activation and how the nuclear metabolite and nucleic acid PAR triggers ALC1 activation is not known.

RESULTS

Modular auto-inhibition in the remodeler ALC1

We set out to investigate what suppresses ALC1 remodeler activity when PARP1 is inactive. Unlike most remodelers, endogenous ALC1 does not purify as a multi-subunit complex and its remodeling activity can be efficiently reconstituted *in vitro* using recombinant protein and DNA, together with PARP1 and NAD^+ (Ahel et al., 2009; Gottschalk et al., 2012; 2009). The enzyme consists of a two-lobed catalytic Snf2-like ATPase domain with homology to ATP-dependent DExx-box helicase (ATPase, **Figure 1A**), which is connected through a linker region of unknown function to a C-terminal macrodomain (macro), which mediates PARP1 activity-dependent chromatin-targeting (Ahel et al., 2009; Gottschalk et al., 2012; 2009).

To establish whether the ALC1 ATPase domain and macrodomain interact, we generated an ATPase fragment (residues 31-615; 'ATPase module') and a fragment containing both linker and C-terminal macrodomain (residues 616-878; 'Macro module') (**Figure 1A**). The domain boundaries were identified using limited proteolysis (**Figure S1**). The recombinant ATPase and macrodomain modules can be expressed individually in *E. coli* and purified to homogeneity. Multiple lines of biochemical evidence show that the two modules form a stable complex. The two fragments interact with each other in pulldown assays (**Figure 1B**). Size-exclusion chromatography assays reveal the formation of a stoichiometric 1:1 complex (**Figure 1C**), which elutes with a molecular size (~138 kDa) close to that of the (near) full-length ALC1 construct (residues 31-878; eluting at ~131 kDa; **Figure 1D**). Thus, ALC1 behaves as a monomer. To determine the affinity of the two ALC1 modules for each other, we employed isothermal titration calorimetry (ITC) assays, which reveal an equilibrium dissociation constant of 96±22 nM (**Figure 1E; Table S1**). These results indicate that the ATPase and macrodomain modules of the ALC1 remodeler engage through a tight, intra-molecular interaction.

To test whether this interaction is also observed in living cells, we used fluorescence-2-hybrid (F2H) analysis (Zolghadr et al., 2012). Tethering of a fluorescent mCherry-LacI-ALC1 macrodomain bait to an integrated LacO array in U2OS cells enriches the eYFP-tagged ALC1 ATPase prey (**Figure 1F; Figure S2**), while unrelated macrodomains do not recognize the ALC1 ATPase. We conclude that in the absence of exogenous DNA damage (i.e. when PARP1 has not been induced), the ALC1 modules specifically interact. Our data suggest that the C-terminal macrodomain of ALC1 packs against one or both of its ATPase lobes in the context of the full-length ALC1 protein, hinting at an intramolecular 'gating' function of the ALC1 macrodomain, as described for the unrelated chromodomain of yCHD1 and the NTR domain of ISWI (Hauk et al., 2010; Ludwigsen et al., 2017). To rule out the possibility that ALC1 may form dimers, trimers or other, higher-order complexes through intermolecular domain-swapping, we conducted co-immunoprecipitation and F2H assays with full-length ALC1 (**Figure S3**). Both assays indicate that ALC1 is a monomer *in vivo* (compared to positive controls).

To probe the domain architecture of ALC1, we used MS-crosslinking. We mapped multiple crosslinks within each of the two ALC1 modules, including between the flexible linker region and the canonical ATPase and macrodomain folds (**Figure S4**). The cross-linking patterns complements well the domain boundaries identified by limited proteolysis (**Figure S1**). The MS-based crosslinks indicate that the ALC1 hinge close to the macrodomain and ATPase, confirming our limited proteolysis data. Together, crosslinking (**Figure S4**) and limited proteolysis (**Figure S1**) hint at a compact arrangement of the ALC1 ATPase and macrodomain modules with respect to each other, consistent with a 'gated' structure, which may restrict DNA access to the ATPase motor. We conclude that in the absence of activated PARP1, intramolecular interactions between the macrodomain and ATPase modules establish an auto-inhibited ALC1 conformation.

PARP1 activation disrupts the auto-inhibited state

ALC1 rapidly recruits to DNA damage sites and massively decompacts chromatin in response to PARP1 activation (Ahel et al., 2009; Gottschalk et al., 2009; Sellou et al., 2016). These activities require a functional, PAR-binding ALC1 macrodomain. We hypothesized that PAR binding to ALC1 may promote an active conformation of ALC1. To determine whether the activation of PARP1 in living cells alters the modular, intra-molecular interactions within ALC1, we used the F2H assay to measure the interaction of the ALC1 ATPase module with the macrodomain prior to and following UV-laser induced PARP1 activation. DNA damage leads to a time-dependent decrease of ALC1 ATPase prey from the tethered ALC1 macrodomain (**Figure 2A**; **Movie S2**). We conclude that PARP1 activation leads to the loss of interaction between the two ALC1 modules. H_2O_2-induced DNA damage also leads to a loss of interaction (**Figure S6A**) and the site of PARP1 activation and ALC1 ATPase–macrodomain dissociation do not need to overlap, since FRAP assays indicate high turnover of our fusion proteins (**Figure S5**). Importantly, a G750E mutant within the macrodomain, which disrupts binding of the pyrophosphate of ADP-ribose in canonical macrodomains (Kustatscher et al., 2005), retains its binding with the ATPase module, even upon PARP1 induction (**Figure S6B**). These data reveal that the interaction between the two ALC1 modules is regulated by PARP1 activation *in vivo*. PAR binding to the macrodomain is coupled to the loss of interaction with the ATPase module, consistent with a direct, allosteric regulation of ALC1 by PAR.

Next, we sought to determine the minimal ALC1 ligand that is necessary and sufficient to trigger ATPase–macrodomain dissociation and PAR-mediated ALC1 activation. We tested the effect of PARP1 activation *in vitro* on the interaction between ALC1 ATPase and macrodomain. Addition of NAD^+ to a pulldown containing PARP1, DNA and the two ALC1 modules disrupts ALC1 ATPase–macrodomain interaction (**Figure 2B**; lanes 4 and 5). Addition of PARP1

inhibitors suppresses the disruptive effect of PARylation on ATPase–macrodomain interaction (lanes 6 and 7 vs. 5). As expected, a point mutant within the ADP-ribose binding pocket of ALC1 (G750E) retains ATPase interaction (lane 8). Consistent with our *in vivo* observations (**Figure S6B**), the interaction of the G750E macrodomain mutant with the ALC1 ATPase resists the addition of PAR (**Figure 2B**; lane 11). In sharp contrast, the wild-type macrodomain dissociates from the ATPase module upon PAR incubation (**Figure 2B**; lane 12). Thus, PAR *in vitro* is sufficient to dissociate the macrodomain of ALC1 from its ATPase. Further, dissociation requires a functional, PAR-binding macrodomain. Thus, PAR allosterically switches ALC1.

Synthetic tri-ADP-ribose is a nanomolar effector of ALC1 allostery

Macrodomains generally show high affinity for monomeric ADP-ribose (Karras et al., 2005; Kustatscher et al., 2005). Our ATPase–macrodomain competition assay, however, reveals that mono-ADP-ribose does not abrogate ALC1 ATPase–macrodomain interactions (**Figure 2B**; lane 10 vs. 12), even when added in 100-fold molar excess, while PAR readily dissociates the complex. Consistently, ITC fails to detect an interaction between mono-ADP-ribose and the wild-type ALC1 macrodomain (**Figure 3A; Table S1**). Although key residues within the canonical ADP-ribose binding pocket of macrodomains are conserved in the PAR-binding ALC1 macrodomain, our data reveal that mono-ADP-ribose is not an ALC1 ligand.

We hypothesized that binding of PAR to ALC1 may require multiple ADP-ribose units. Interestingly, a PAR footprinting assay revealed that ALC1 protects oligomers of ~3 to >20 ADP-ribose units in length (Gottschalk et al., 2012). We therefore synthesized dimeric and trimeric forms of ADP-ribose (Kistemaker et al., 2015) and tested their binding to the ALC1 macrodomain. Remarkably, the ALC1 macrodomain binds di-ADP-ribose with a K_D = 3.7 μM and tri-ADP-ribose with nanomolar affinity (K_D = 10.6 nM; **Figure 3A; Table S1**). Extending

ADP-ribose from monomer to trimer thus turns the NAD metabolite into a high-affinity ligand. In sharp contrast to ALC1, the macrodomain of human histone macroH2A.1.1 recognizes mono-ADP-ribose and di-ADP-ribose with the same K_D (**Figure S7**). This indicates that ALC1 contains a NAD^+-metabolite binding surface that recognizes multiple features within tri-ADP-ribose. Highlighting the high affinity of the ALC1 macrodomain for tri-ADP-ribose, thermal shift assays reveal a ~10 ºC stabilization of the macrodomain by tri-ADP-ribose (**Figure S8A**). To probe the selectivity of ALC1 toward related nucleotides, we conducted ITC with tri-adenylate RNA, tri-adenylate ssDNA and penta-adenylate ssDNA. All fail to bind ALC1 (*data not shown*). Further, ITC and size-exclusion chromatography assays reveal a 1:1 complex between the ALC1 macrodomain module and tri-ADP-ribose (**Figures 3A** and **S8B**). ALC1 seems unique among known macrodomain proteins in showing strong preference for oligo-ADP-ribose.

Our quantitative assays show that the ALC1 macrodomain reads only oligomers of ADP-ribose. It can thus discriminate PAR and PARylation from other monomeric NAD metabolites and mono-ADP-ribosylated proteins. We hypothesize that the second and third ADP-ribose units of tri-ADP-ribose mediate additional contacts, which extend beyond the canonical ADP-ribose binding pocket of macrodomains, consistent with PAR footprinting data (Gottschalk et al., 2012).

Tri-ADP-ribose releases ALC1's auto-inhibition and triggers remodeler activation

The ability of synthetic tri-ADP-ribose to bind the ALC1 macrodomain with nanomolar affinity and high selectivity gives us a useful chemical probe to dissect the allosteric regulation activation of ALC1 enzyme activity. We thus tested whether di- and tri-ADP-ribose can mimic PAR at the functional level and are able to disrupt ATPase–macrodomain interactions *in vitro*. V5-based pulldowns assays with tagged ALC1 macrodomain in complexed to with untagged ATPase domain show that the addition of di-ADP-ribose does not detectably affect the

interactions (**Figure 3B**; lane 5 vs. 3). In contrast, addition of the nanomolar tri-ADP-ribose ligand (in 2.5-fold molar excess) clearly disrupts interactions between the two ALC1 modules (**Figure 3B**; lane 6 vs. 3). Importantly, neither polyA-DNA, nor tri-ADP-ribose added to a PAR-binding deficient G750E macrodomain mutant, cause macrodomain dissociation from the ALC1 ATPase domain (**Figure 3B**; lanes 7-9 vs. 3). This indicates that trimeric ADP-ribose is sufficient to disrupt the intermolecular association between the two ALC1 modules. To quantitate the change in affinity between the two domains in the presence of the tri-ADP-ribose ligand, we conducted ITC titrations assays of the ALC1 macrodomain with the ALC1 ATPase domain in the presence and absence of equimolar tri-ADP-ribose concentrations. We observed a reduction inTri-ADP-ribose reduces the affinity between the two ALC1 domains modules from ~70 nM to below the detection limit (the extrapolated K_D is = >50 μM; **Figure 3C; Table S1**). These data indicate that tri-ADP-ribose thus reduces the affinity of the ALC1 macrodomain for its the ATPase domain by at least 3 or more orders of magnitude. While we have been unable to obtain larger longer forms of ADP-ribose oligomers (e.g. tetra-ADP-ribose), we conclude that the the tri-ADP-ribose probe effector molecule reproducesis an effective PAR mimic. a salient structural and functional feature of the physiological PAR ligand of ALC1.

Next, we sought to determine the relevance of tri-ADP-ribose binding on the catalytic activity of the ALC1 remodeler. We established a robust, DNA-dependent ATPase assay for both the ALC1 ATPase module (31-673) and the (near) full-length ALC1 protein (31-878). We find that the ALC1 ATPase module shows robust, DNA-dependent ATPase activity (**Figure S9**). Importantly, titration of the ALC1 macrodomain module to the ALC1 ATPase lowers ATPase activity (**Figure S9A**). Once a 2.5 molar excess of ALC1 macrodomain is added to the ALC1 ATPase, the resulting complex is inactive, revealing background ATPase activity similar to that

of the ALC1 ATPase without DNA. The ALC1 macrodomain thus represses the inherent ATPase activity present in the ALC1 ATPase module.

To test whether the addition of monomeric ADP-ribose alters the ATPase activity of ALC1, we added monomeric ADP-ribose to the ATPase–macrodomain complex. A 6-fold molar excess of mono-ADP-ribose fails to rescue ATPase activity (**Figure 3D**). Crucially, addition of a 2-fold excess of tri-ADP-ribose robustly de-represses the ALC1 ATPase, going from <2% without tri-ADP-ribose, to ~60% of the activity of the free ALC1 ATPase module (**Figure 3D**). Thus, tri-ADP-ribose binding to the ALC1 ATPase–macrodomain complex strongly activates the ALC1 remodeler. In addition, we tested whether tri-ADP-ribose alters the activity of (near) full-length ALC1. As expected, the ATPase activity of this construct is low, including in the presence of mono-ADP-ribose. However, tri-ADP-ribose strongly activates ALC1 (**Figure 3E**). The level of activation induced by tri-ADP-ribose is lower than in our assays using the reconstituted ALC1 complex. However, this is likely the result of degradation products present in our recombinant ALC1 (31-878) construct (**Figure S9B**). Indeed, some of the proteolytic fragments observed in our ALC1 construct (31-878) may lack (parts of) the inhibitory macrodomain and display ATPase activity in the absence of the trigger tri-ADP-ribose. Together, our assays show that tri-ADP-ribose is a potent activator of the DNA-dependent ATPase activity of the ALC1 remodeler.

The NAD mMetabolite tri-ADP-ribose -inducesd allosteric conformational changes within ALC1ungating

Our assays indicate that tri-ADP-ribose may act as an allosteric trigger of the conformation and enzymatic activity of the ALC1 enzyme. To investigate how tri-ADP-ribose alters the structure of full-length ALC1, we used MS-coupled H/D-exchange (HDX) measurements (HDX) monitored by mass spectrometry (HDX-MS) to identify regions within the ALC1 where the hydrogen

bonding of the amide groups of the protein backbone change upon tri-ADP-ribose addition of tri-ADP-ribose. Peptide segments resulting from pepsin proteolysis of ALC1, collectively covering 82.2% of the full-length sequence, were analyzsed and used to resolve the HDX of local regions of ALC1 regions in the presence and absencewith and without of tri-ADP-ribose (**Figures 4A and** ; **Figures S107-S129**). Upon binding tri-ADP-ribose, HDX-MS revealsed increases in HDX corresponding to an increase in dynamics and a destabilization of the hydrogen H-bonding in distinct regions of ALC1. First, we observed changes within two neighboring segments encompassing a predicted α-helix in the ALC1 macrodomain, which lies in immediate proximity to the canonical mono-ADP-ribose ligand binding site (residues 832-858; HDX3; **Figure 4B**). This is consistent with the binding of tri-ADP-ribose within and near the canonical macrodomain pocket, leading to an altered hydrogen H-bonding environment of for residues either involved in (tri-) ADP-ribose interaction and/or intramolecular ALC1 contacts. Remarkably, tri-ADP-ribose binding to the ALC1 macrodomain also changes the HDX pattern of residues which are located in lobe 2 of the Snf2 ATPase fold, specifically residues 319-357 (HDX1) and residues 392-415 (HDX2; **Figure 4A,B**). This indicates that the binding of tri-ADP-ribose to the macrodomain module of the ALC1 remodeler is associated with concerted changes in the hydrogen H-bonding of regions in lobe 2 of the ATPase domain (**Figure 4B**).

Interestingly, in yeast yChd1 and ISWI, the a related surface of the ATPase lobe 2 directly contacts the protein's chromodomain 1 domain and NTR region, respectively. This allows these remodelers to physically gate access of DNA to the ATPase motor. Our H/DHDX-MS data reveal a destabilization of hydrogen H-bonding and increased dynamics of lobe 2 within the ALC1 ATPase motor upon the binding of the PAR mimic tri-ADP-ribose. Moreover, we observe tri-ADP-ribose- induced changes also near the canonical ADP-ribose binding pocket of the ALC1 macrodomain. We conclude that the HDX-MS data thus identifyies regions of the ALC1 protein

that undergo hydrogen H-bond destabilization and conformational gating upon the tri allosteric activation induced by tri--ADP-ribosee-induced allosteric activation. We hypothesize that PAR binding to the ALC1 macrodomain may grant access of the ATPase motor to nucleosomal DNA-containing substrates, switching ALC1 into an 'ungated' conformation capable thatof hydrolyzesing ATP and slidesing nucleosomes.

Somatic cancer mutants drive the ungating and activation of ALC1

To test whether the surface regions identified by in our HDX-MS assays are important for the intramolecular interactions and enzymatic regulation of in ALC1, we engineered point mutants in the HDX1, HDX2 and HDX3 regions of ALC1 and tested for how they affect the effect of these mutations in ATPase–macrodomain interaction our using *in vivo* F2H assays ATPase–macrodomain interaction assay (**Figure 4C**). To increase the dynamic range of our assay, we used an the ALC1 macrodomain G750E mutant, which that binds PAR with lower affinity, as a reference. Mutating distinct residues located within the HDX1 and HDX2 region of the ALC1 ATPase all strongly reduce or abrogate binding to the ALC1 macrodomain module (**Figure 4C**). In turn, a single point mutation in HDX3 (R857E) is sufficient to disrupt ATPase–macrodomain interaction, while other point mutants outside of the identified HDX regions do not alter the interaction between the two ALC1 modules (**Figure 4C**). Interestingly, residues R857, R842 and R860 is are mutated in human gliomas [somatic cancer mutant; (Bamford et al., 2004)(Bamford et al., 2004)], raising the possibility that a destabilization of the interactions between the HDX3 region in the ALC1 macrodomain and the ATPase module may contribute to cancer. Indeed, the cancer SNPs R875Q and R842H/R860W, when introduced into the ALC1 macrodomain, reduce interaction with the ALC1 ATPase (**Figure 4C**). Similarly, point mutants within HDX1 and HDX2 of the ATPase module reduce ALC1 ATPase–macrodomain

interactions (**Figure 4C**). We conclude that ALC1 identified surface regions in ALC1 that alter identified in our HDX-MS patterns analysis upon addition of tri-ADP-ribose are important in regulatingcontribute to the intramolecular ATPase–macrodomain interactions. We suggest thatB the HDX and F2H data provide insight into how the binding of tri-ADP-ribose to ALC1 disrupts the intramolecular contacts that are critical for for ALC1's in its auto-inhibitioned state.

Engineering of aA tethered ALC1 ATPase module remodels constitutively activechromatin *in vivo* ALC1 remodeler

The ability of PAR to activate ALC1 through by the releasereleasing of the interaction between of ALC1's ATPase and from the macrodomain modules predicts that ALC1 ATPase fragments lacking the PAR-regulated macrodomain might display remodel chromatin *remodeling in vivo* activity in the absence of DNA-damage inducedwithout requiring PARP1 hyperactivation. Since PARP1 activation *in vivo* leads to the massive relaxation of chromatin structure upon DNA damage and ALC1 is a key remodeler in mediating this chromatin plasticity (Sellou et al., 2016)(Sellou et al., 2016), we decided to use an *in vivo* chromatin relaxation assay to test a range of engineered ALC1 macrodomain-deletion fragments.

Since ALC1 does not recruit to chromatin upon DNA damage in the absence of its PAR-binding macrodomain, we tethered full-length, fragment and mutant LacI-ALC1 fusions constructs to an chromatinised, integrated LacO array in human cells. As expected, wild-type, full-length ALC1 (residues 1-897) does not alter the appearance of the LacO-array when tethered to it (**Figure 5A**). In sharp contrast, when the C-terminal macrodomain of ALC1 is deleted, the remaining ALC1 protein fragment (residues 1-673) strongly decompacts the LacO array (**Figure 5A**). This chromatin relaxation is seen with other ALC1 C-terminal fragments, but not in a fragment as short as (1-615). This indicates that sequences within linker II of ALC1 (residues

616-673; **Figure 1A**) contribute to ALC1's its chromatin remodelling activity, while the macrodomain is inhibitory to ALC1 chromatin reorganizationremodeling activity *in vivo*. Importantly, mutation of conserved residues within the ALC1 helicase-like motifs of the ATPase, which that disrupt either disrupt ATP binding, DNA binding or ATPase activity in the Snf2-like ATPase domain of ALC1 (Ahel et al., 2009; Gottschalk et al., 2009)(Ahel et al., 2009; Gottschalk et al., 2009), strongly reduces or abolishes ALC1-induced chromatin relaxation (**Figure 5A**). We conclude that we have thus Tetheredengineered constitutively active ALC1 fragments lacking the macrodomain that thus possess ATPase and remodeling activity in the absence of DNA damage induction and PARP1 hyperactivation.

Next, we tested whether the macrodomain of ALC1 alters the inherent remodeling activity of the LacI-tethered ALC1 ATPase when added *in trans*. Addition of the ALC1 macrodomain module to the active, tethered ALC1 ATPase reduces chromatin decompaction (**Figure 5A**). This inhibition is enhanced by a PARP1 inhibitor, indicating that the PAR-free ALC1 macrodomain represses ALC1 activity *in vivo*.

Next, we tested whether cancer SNPs within HDX3, which cause a loss-of-interaction between the ALC1 ATPase and macrodomain (Figure 4C), alters ALC1 remodeling activity when introduced in the context of full-length ALC1 tethered to LacO via LacI. Interestingly, the point mutants ALC1 R875Q and R842H/R860W are as active in remodeling as the constitutively active ALC1 ATPase module (**Figure 5A**). Furthermore, FCS assays show that the diffusion behavior of wild-type ALC1 (fast) differs from that of the HDX1 and HDX3 mutants (slower; **Figure S13**), suggesting that HDX mutants disrupt intramolecular ATPase–macrodomain interactions promote an ungated structure and may bind DNA. The Our data indicate that the ALC1 macrodomain inhibits the ATPase activity of ALC1 at physiological levels of PARP1 activity. In its absence, a tethered ALC1 ATPase module readily remodels chromatin *in vivo*.

Further, cancer mutants phenocopy the constitutively active ALC1 ATPase fragment. Our tethering assay reveals that somatic cancer mutations in the ALC1 oncogene disrupt inhibitory intramolecular interactions in ALC1 (**Figure 4C**) and lead to a constitutively active remodeling enzyme (**Figure 5A**). This may be relevant to cancer.

Modular allostery in ALC1 regulates interaction with PARP1

Allostery describes how effector molecules alter the active site of an enzyme. Our data indicate that the ALC1 macrodomain module acts as a 'gate', promoting an inactive conformation in the absence of its PAR ligand. Binding of the effector NAD^+-metabolite PAR to the macrodomain module opens the conformation of ALC1 in a concerted or sequential manner, which activates the remodeling activity. To further probe the PAR-regulated modular allostery in ALC1, we tested whether, in turn, the ATPase module affects the ability of the ALC1 macrodomain to recognize its effector molecule, PARylated PARP1, in living cells. While full-length ALC1 does not readily interact with full-length PARP1 in untreated human cells using the F2H assay (**Figure 5B** and **Movie S3**), DNA damage induction with H_2O_2 promotes the interaction between these two proteins, consistent with the recognition of activated, PARylated PARP1 by ALC1. Interestingly, an ALC1 fragment lacking the catalytic ATPase domain readily interacts with PARP1, even in the absence of exogenous DNA damage (**Figure 5B**). Treatment of cells with a PARP1 inhibitor abrogates the this interaction. This indicates that the isolated macrodomain module of ALC1 recognizes ADP-ribosylated forms of PARP1 under 'non-DNA-damage' conditions (**Figure 5B**), likely reflecting background ADP-ribosylation levels of PARP1. Consistently with this interpretation, a point mutant in the ALC1 macrodomain that abolishes reduces PAR binding (G750E), or mutation of a key residue in PARP1 (E988K) that is responsible for the elongation of mono-ADP-ribosyl-ated PARP1 to poly-ADP-ribosyl-ated PARP1, disrupts the interaction

between the ALC1 macrodomain and PARP1 (**Figure 5B**). We conclude that the isolated ALC1 macrodomain module interacts with ADP-ribosylated PARP1 under physiological conditions, while full-length ALC1 requires a high threshold of DNA-damage and PARP1 hyperactivation to in order to interact with PARylated PARP1. Thus, the ATPase module of ALC1 lowers the affinity of the macrodomain for PARylated-PARP1, consistent with modular allostery. The observation that full-length ALC1 only interacts with PARP1 in cells when its activity has been strongly induced by DNA damage (**Figure 5B**) reveals reciprocal inhibitory interactions within ALC1 (**Figure 5C**; *left*), while the isolated ALC1 macrodomain interacts with PARP1 even in the absence of exogenous DNA damage. We suggest that the modularity these of ALC1 features allows ALC1 the remodeler to be activated only only once a threshold of PARP1 induction activitation has been reached.

. Our data indicate that the modular structure of ALC1 allows the remodeler's recruitment to chromatin and its remodeling activity to be exquisitely regulated by PARP1 hyperactivation (**Figure 5C**). Our *in vitro* and *in vivo* assays indicate that the interaction of its macrodomain module with the ATPase module is responsible for ALC1's autoinhibited state in the absence of exogenous DNA damage. Binding of the effector PARylated-PARP1 to ALC1 triggers the direct de-repression through a physical 'ungating' of the ATPase module, as our HDX data indicate. This ligand-induced de-repression is reminiscent of allosterically regulated signaling enzymes, such as autoinhibited receptor tyrosine kinases. Additional features in ALC1 include a stretch of positively charged residues (616-673) within the Linker 2 region of ALC1's macrodomain module, which are required for *in vivo* chromatin remodelling (**Figure 5A**) and that cross-link the core macrodomain to a region that shows changes in HDX properties upon addition of tri-ADP-ribose (HDX3; **Figure 4A**). This suggests that PAR binding to the ALC1 macrodomain may displace a positive regulatory fragment, allowing it to contribute to enzyme activity. Finally, the

linker region of ALC1 can be ADP ribosylated *in vitro* (Zhang et al., 2013). Likely, such modifications would lead to conformational changes within ALC1, including an auto-inhibited structure, similar to what has been reported for acetylation in the remodeler RSC4 (VanDemark et al., 2007). Upon DNA-damage, PARP1 activation generates a sufficient amount of PARylated-PARP1 to compete for binding to the macrodomain of ALC1. In turn, the binding of a minimum of three ADP-ribose units to ALC1's macrodomain effectively releases the catalytic domain, 'ungating' the inhibited enzyme and allowing it to interact with nucleosomal templates to effectively remodel chromatin.

DISCUSSION

Auto-inhibitory interactions play important roles in cell signaling and in the regulation of the activity of chromatin and repair factors (DaRosa et al., 2015; Guo et al., 2015)(DaRosa et al., 2015; Guo et al., 2015). Considering the emergent role of remodelers in cancer (St Pierre and Kadoch, 2017; Zhao et al., 2017)(St Pierre and Kadoch, 2017; Zhao et al., 2017), a better understanding of how DNA damage alters chromatin structure is important. While the mechanisms that ALC1 and CHD2 employ to relax chromatin structure upon DNA damage *in vivo* (**Movie S1**) are not known, and the remodelers' substrate(-s) *in vivo* remain to be identified, here we have identified and dissected the mechanisms that allow the human oncogene ALC1, which massively decompacts chromatin upon PARP1 activation, to be exquisitely tighly regulated by the cellular NAD^+ metabolite PAR (**Figure 6**). . We show that reciprocal interactions between the ALC1 ATPase module and its macrodomain establish an allosteric mechanism that tightly controlsallow ALC1 activity to be controlled by ation upon PARP1 activation. We find thatT the binding of an oligomer of at least three ADP-ribose units to ALC1's macrodomain induces triggers conformational changes that disrupt auto-inhibitory interactions within ALC1. This 'ungates' the ATPase module, (**Figures 3, 4**)promoting DNA-dependent ATPase activity *in vitro* and remodeling *in vivo* (**Figures 3-5**). This Mmodular allostery thus ensures that ALC1 is exquisitely sensitive to and specific selective to for oligomeric forms of ADP-ribose, which likely are only produced upon significant PARP1 activation. under acute DNA damage conditions. We infer that PAR acts as a catalytic trigger only once a threshold of PARP1 induction has been reached. While modular allostery is well described in the signaling field,M most 'reader' modules in chromatin biology and epigenetics are thought to play primarily a recruitment and tethering function. Our identification of a reciprocal regulation interaction between a PAR-binding macrodomain ('reader' module) and the catalytic ATPase module of

ALC1 suggest that such types of regulation could be more widespread, addsing to the allostery described for DNA methyltransferases (Guo et al., 2015; Jeltsch and Jurkowska, 2016)(Guo et al., 2015; Jeltsch and Jurkowska, 2016).

In vivo, binding of the PAR effector to the macrodomain occurs when ALC1 recruits to DNA damage sites, which tethers the remodeler to chromatin and allows ALC1 to remodel and greatly relax chromatin structure. Our study does not identify the specific, physiological substrate that ALC1 remodels on chromatin. Indeed, Swi2/Snf2 remodelers such as Mot1 remodel non-nucleosomal substrates (Wollmann et al., 2012)(Wollmann et al., 2012). *In vivo*, the PARylation of histones, PARP1, ALC1 and/or other chromatin factors may thus all contribute to the how observed ALC1 -catalyzesd chromatin relaxation.

The selectivity of ALC1 toward oligo-ADP-ribose and the fact that the enzyme's ATPase impairs the ability of ALC1's macrodomain to bind PARP1 under non-DNA damage conditions (**Figure 5B**), likely helps to ensure that robust, PARP1-dependent chromatin relaxation is only catalyzed once PARP1 has been activated, such as during DNA damage. Our data reveal how the activity of a remodeler is gated by the PARP1-product and nucleic acid PAR through regulatory interactions mediated by ALC1's macrodomain. This adds to our mechanistic understanding of how remodelers are regulated through gating mechanisms. In the special case of ALC1, this occurs through a cellular NAD^+ metabolite, which actsing as an allosteric trigger of the de-repression mechanism and , which is mediated by the remodeler's core macrodomain fold and additional contacts with the PAR ligand..

OFurther, our analysis also identifies avenues of how the oncogene ALC1 might be targeted to in treat cancer. Small molecules that inhibit its allostery or activity should reproduce *ALC1* knockdown phenotypes, such as reduced tumor growth, reduced reprogramming and increased sensitivity to chemotherapy eutics (Jiang et al., 2015; Liu et al., 2016)(Jiang et al., 2015; Liu et al., 2016). Compounds that stabilize the inactive, 'gated' conformation of ALC1, that which lower its catalytic activity, or that disrupt its ability to recognize PAR, should suppress the unusual but potent chromatin relaxation activity of this human oncogene.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Experimental Procedures, nine figures, and one table.

AUTHOR CONTRIBUTIONS

H.R.S., M.H., G.T. and A.G.L. conceived the project. H.R.S. performed most of the experiments. A.P.N. conducted ITC assays,and G.K. conducted ATPase assays, I.R.M. worked with H.R.S. on the HDX-MS measurements, H.A.V.K. synthesized di-meric and tri-meric ADP-ribose, M.H. and G.K. determined complex association between ATPase and macrodomain module by gel-filtration, N.H. conducted X-linking MS assays, S.E. provided full-length ALC1 protein, C.H. helped with molecular biology and S.H. assisted with live-cell imaging. H.R.S., I.R.M. and K.D.R. analysed HDX datasets. H.R.S. and A.G.L. wrote the manuscript. All authors discussed the work and reviewed the manuscript.

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FIGURE LEGENDS

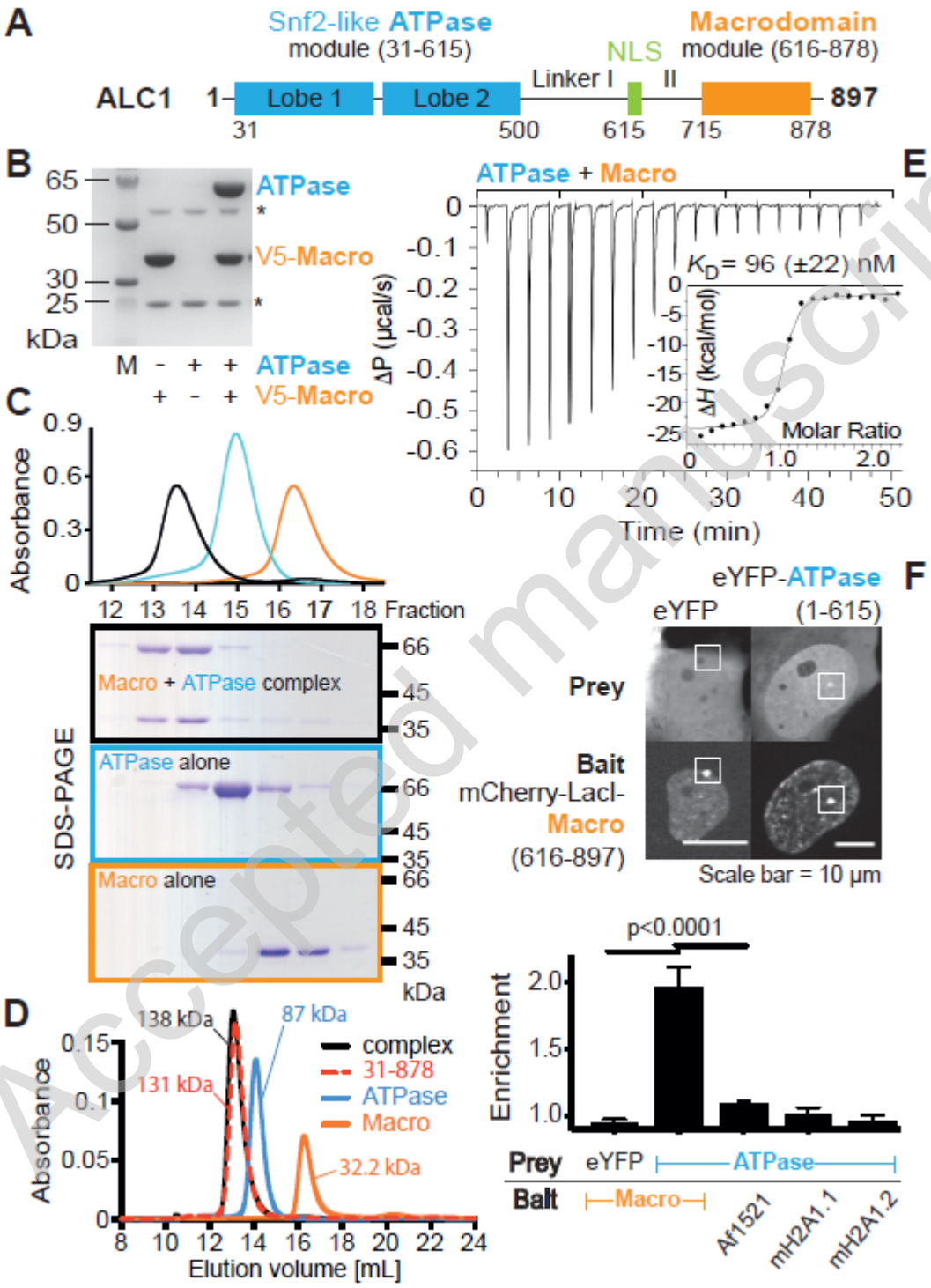

Figure 1. The PARP1-dependent chromatin targeting module ofmacrodomains of ALC1 folds back and tightly interacts with the Snf2-like ATPase domain in the enzyme's inhibited state

(A) The human ALC1 oncogene is composed of two primary modules: an N-terminal Snf2-like ATPase module (residues 31-615) and a C-terminal macrodomain module (616-878). The boundaries were defined using limited proteolysis (Figure S1). NLS = nuclear localization signal.

(B) SDS-PAGE of a V5-based pulldown with recombinant, purified ALC1 macrodomain and ATPase module. The asterisk denotes anti-V5 IgG heavy and light chains.

(C) Size exclusion chromatography (SEC) of recombinant, purified ALC1 macrodomain (residues 636-878,orange), ATPase domain (residues 31-615, cyan) and *in vitro* a complex reconstituted *in vitro* complex by mixing the macrodomain and ATPase module in equal molar ratios (black), plus SDS-PAGE of the eluted fractions..

(FD) Comparison of the elution profiles by gel filtration of the reconstituted ALC1 ATPase-–macrodomain complex with purified, near full-length ALC1 (residues 31-878).The ALC1 ATPase module and ALC1 macrodomain module tightly interact with each other in the absence of activated PARP1 enzyme. MS crosslinking data for the full-length protein reveal interactions between a core portion of the linker region (Linker) and surfaces within each of the globular ATPase and macrodomain modules of ALC1 (*blue lines*; see Figure S3).

(DE) Isothermal titration calorimetry (ITC) assays show that ALC1's two modules bind each other in a high affinity, exothermic reaction and with 1:1 stoichiometry ($N = 0.87 \pm 0.05$).

(EF) Fluorescence-2-hybrid (F2H) analysis in live human cells (Zolghadr et al., 2012)(Zolghadr et al., 2012) reveals that ALC1's ATPase module (eYFP-ATPase; *prey*) readily enriches on a LacO-array tethered mCherry-LacI-macrodomain construct (*bait*). Example image (*top*), as well as quantitation (n = 20) and comparison with unrelated macrodomain fusion proteins to reveal the

specificity of the ALC1 ATPase and ALC1 macrodomain interaction (*bottom*). (F) The ALC1 ATPase module and ALC1 macrodomain module tightly interact with each other in the absence of activated PARP1 enzyme. MS crosslinking data for the full-length protein reveal interactions between a core portion of the linker region (Linker) and surfaces within each of the globular ATPase and macrodomain modules of ALC1 (*blue lines*; see Figure S3).

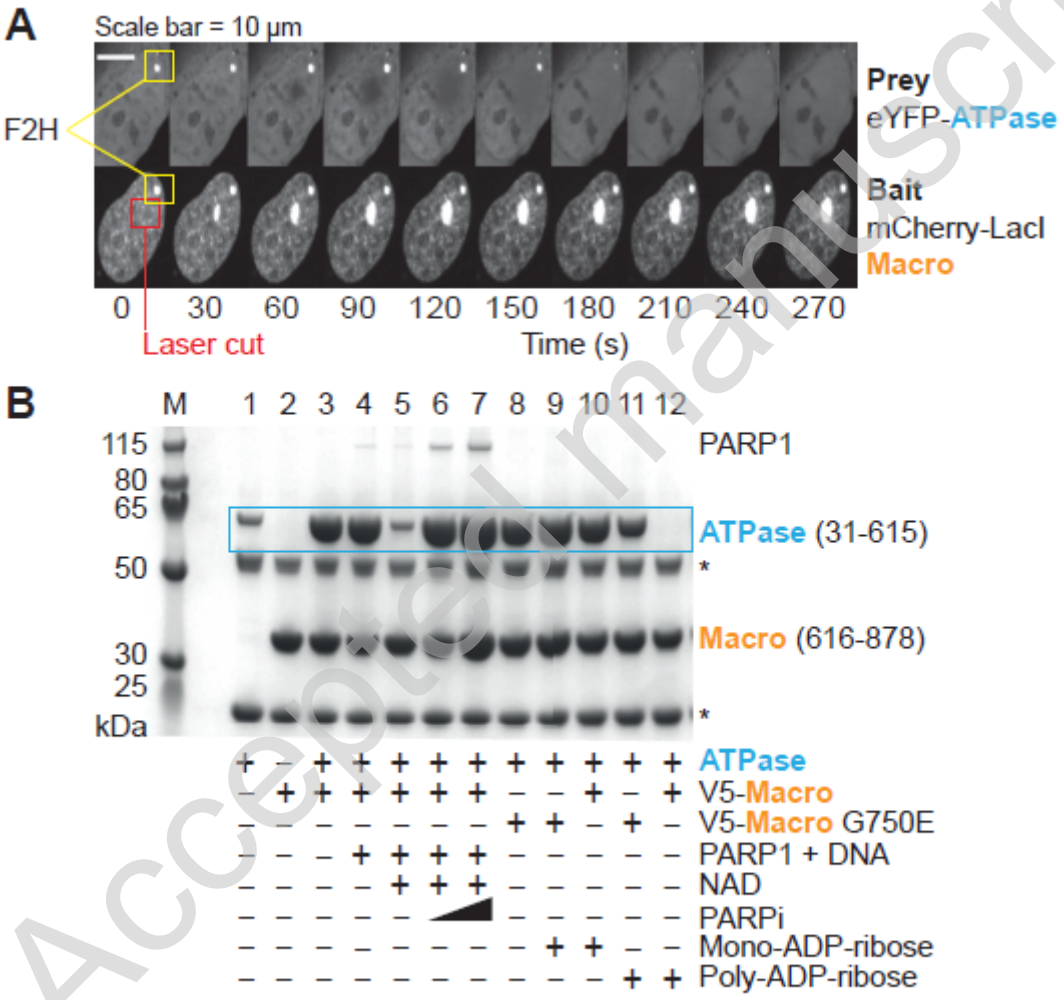

Figure 2. Acute DNA damage and PARP1 activation trigger the release of the ALC1 ATPase module from a tethered ALC1 macrodomain

(A) The LacO-tethered LacI-ALC1 macrodomain module (bait) enriches ALC1's ATPase (prey) in the absence of exogenous DNA damage (*left*, compare white dot within the top and bottom *yellow squares*). Upon targeted and localized UV-laser-induced DNA damage (*red square*), the activation of the endogenous PARP1 enzyme leads to the dissociation of ALC1's ATPase module from the chromatin-tethered macrodomain (next panels). Upon DNA damage, the ALC1 macrodomain bait enriches at the DNA damage site, as expected from the local synthesis of its ligand poly-ADP-ribose, PAR (Ahel et al., 2009; Gottschalk et al., 2009)(Ahel et al., 2009; Gottschalk et al., 2009). Both ALC1 recruitment (Ahel et al., 2009; Gottschalk et al., 2009)(Ahel et al., 2009; Gottschalk et al., 2009) and the disruption of ATPase–macrodomain interactions require PARP1 activity (Figure S5).

(B) *In vitro* pulldown assays with V5-tagged ALC1 macrodomain reconstitute the PARP1 activity- and PAR-dependent dissociation of the modular ATPase–macrodomain interaction. Lanes 1 and 2 with untagged ATPase and V5-tagged macro modules alone, respectively. Disruption of the ATPase–macrodomain complex requires PARP1, DNA and cofactor NAD^+ (lanes 3-5). Addition of small-molecule PARP1 inhibitors suppresses the PARP1 activity-dependent dissociation (lanes6 and 7). The ALC1 ATPase–macrodomain complex is wholly disrupted by addition of pure PAR to the reaction, while a macrodomain point mutant (G750E), which alters ADP-ribose binding within its canonical ADP-ribose binding pocket, largely retains binding to the ALC1 ATPase module (lanes 11 and 12). In contrast to PAR, monomeric ADP-ribose fails to disrupt ATPase–macrodomain interactions for both wild-type and G750E mutant ALC1 macrodomain module (lanes 8-10). The asterisk denotes anti-V5 IgG heavy and light chains.

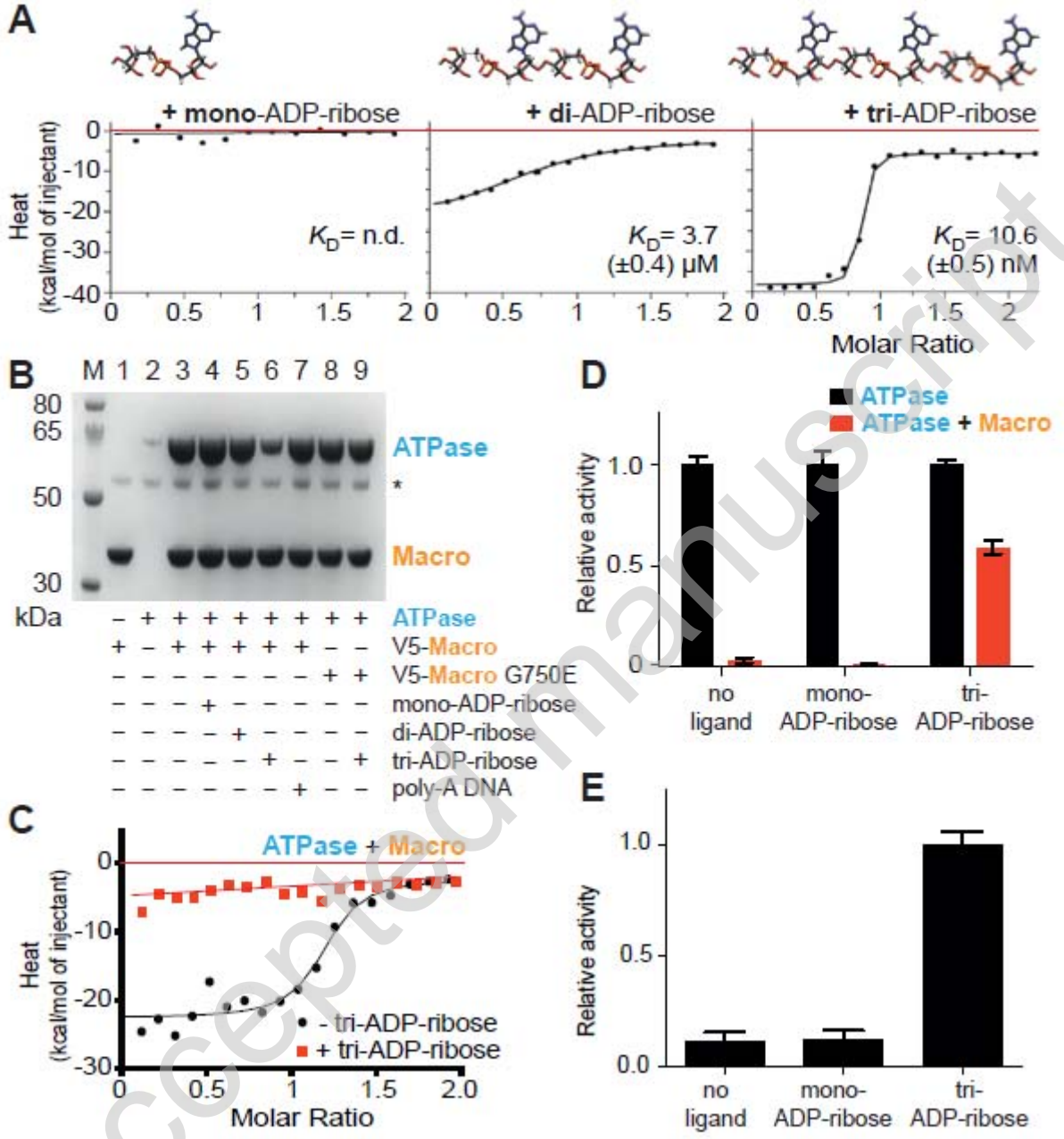

Figure 3. Tri-ADP-ribose is a nanomolar effector of ALC1 that disrupts the intramolecular ALC1 ATPase–macrodomain module interaction

(A) ITC binding isotherms between the ALC1 macrodomain and mono-, di- and tri-meric, dimeric and trimeric ADP-ribose ligands. The Wiseman plot was not baseline-subtracted to account for the heat-of-dilution of the nucleotide ligands.

(B) SDS-PAGE gel of a V5-tagged ALC1 macrodomain pulldown with ALC1's ATPase module. Addition of tri-ADP-ribose disrupts the interaction (lane 6 vs. 3-5). In contrast, an ADP-ribose-binding pocket mutant (G750) suppresses the ability of tri-ADP-ribose to compete off the ALC1 ATPase module. Abrogation of the ATPase–macrodomain module interaction by tri-ADP-ribose thus requires an intact ADP-ribose binding pocket in the ALC1 macrodomain. The asterisk denotes anti-V5 IgG heavy chain.

(C) ITC binding isotherm for the interaction between the ALC1 macrodomain and ATPase module in the presence (*red squares*) and absence (*black circles*) of tri-ADP-ribose. The Wiseman plot was not baseline-subtracted.

(D) Tri-ADP-ribose activates the inactive ALC1 ATPase-–macrodomain complex. The DNA-dependent ATPase activity of the ALC1 ATPase module was measured using a malachite green assay in the presence and absence of a 2.5 molar excess of ALC1 macrodomain module, as well as in the absence (*left*) or presence of either a 6-fold molar excess of ADP-ribose (*middle*) or a 2-fold molar excess of tri-ADP-ribose (*right*). The data are normalized to the respective mean activity of the ATPase module alone (*black bars*; *n*=3).

(E) Tri-ADP-ribose activates the ALC1 remodeler. The near full-length ALC1 construct (31-878) shows only basal ATPase activity in the absence or the presence of a 15-fold molar excess of ADP-ribose. In contrast, a 5-fold molar excess of tri-ADP-ribose greatly stimulates ALC1-catalyzed and dsDNA-dependent ATP hydrolysis. The data are normalized to the mean value of ALC1 activity in the presence of tri-ADP-ribose (*n*=3).

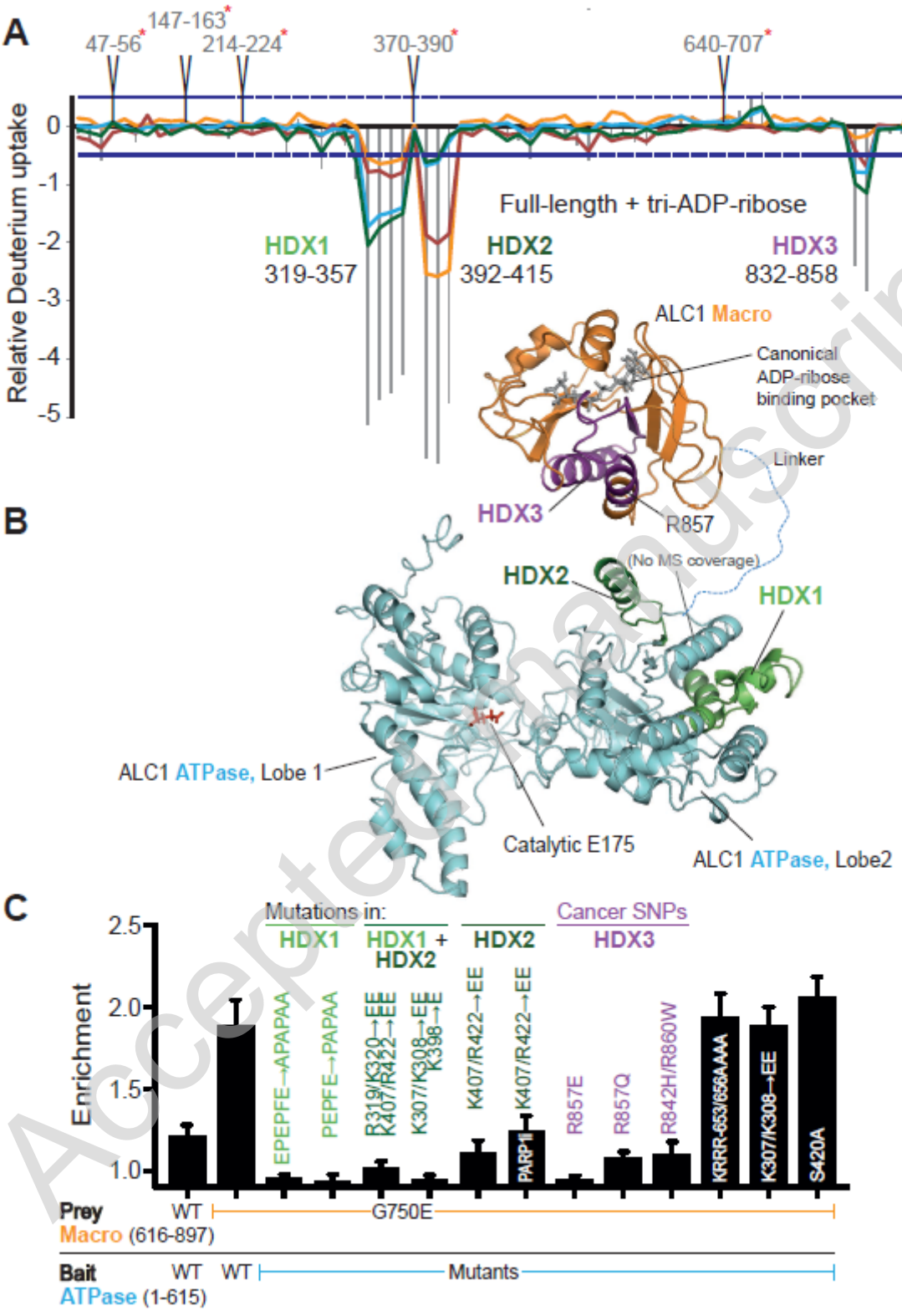

Figure 4. Ligand-induced ungating of the auto-inhibited ALC1 remodeler

(A) HDX-MS analysis reveals concerted destabilization of hydrogen H-bonding in ALC1 upon tri-ADP-ribose binding. Increased HDX in peptides located in lobe 2 of the ALC1 ATPase (regions HDX1 and HDX2; residues shown) and surrounding the canonical ADP-ribose binding pocket of the ALC1 macrodomain (HDX3). Negative values indicate increased H/D uptake upon ligand binding. Asterisks indicate ALC1 regions that were not resolved by MS (*grey;* see also Figure S7). Samples were incubated with D_2O for 0.25 min (*orange*), 1 min (*red*), 10 min (*blue*) and 60 min (*green*). Summed deuterium uptake over the measured time points (*gray bars*). The difference in H/D exchange was considered significant if >0.5 (*blue dashed line*), corresponding to a 98.75% confidence interval; $n = 3$.

(B) HDX results for ALC1 in the presence or absence of tri-ADP-ribose shown on I-TASSER (Roy et al., 2010)(Roy et al., 2010) structural models of the ALC1 macrodomain and ATPase. Peptides that show a difference in HDX upon addition of tri-ADP ribose are colored purple in the macrodomain (*top*, HDX3) and green/lime in the ATPase (*bottom*, HDX1 and HDX2). The linker connecting the ATPase and macrodomain is shown as a dotted line (*right*). Its structure is not known and it is largely not covered by our HDX data. Residues shown include R857 within the macrodomain's HDX3 region, a residue whose mutation is implicated in human gliomas, and the catalytic E175 in the ATPase as a reference for ALC1's active site.

(C) Mutational analysis of HDX1, HDX2 and HDX3 regions inusing our the F2H-based-based ALC1 ATPase–macrodomain –ATPase module interaction assay. We targeted residues within the HDX regions that contained patches of negative or positively charges, including the cancer SNPs (R85Q, R842H & R860W).including a cancer residue (R857).

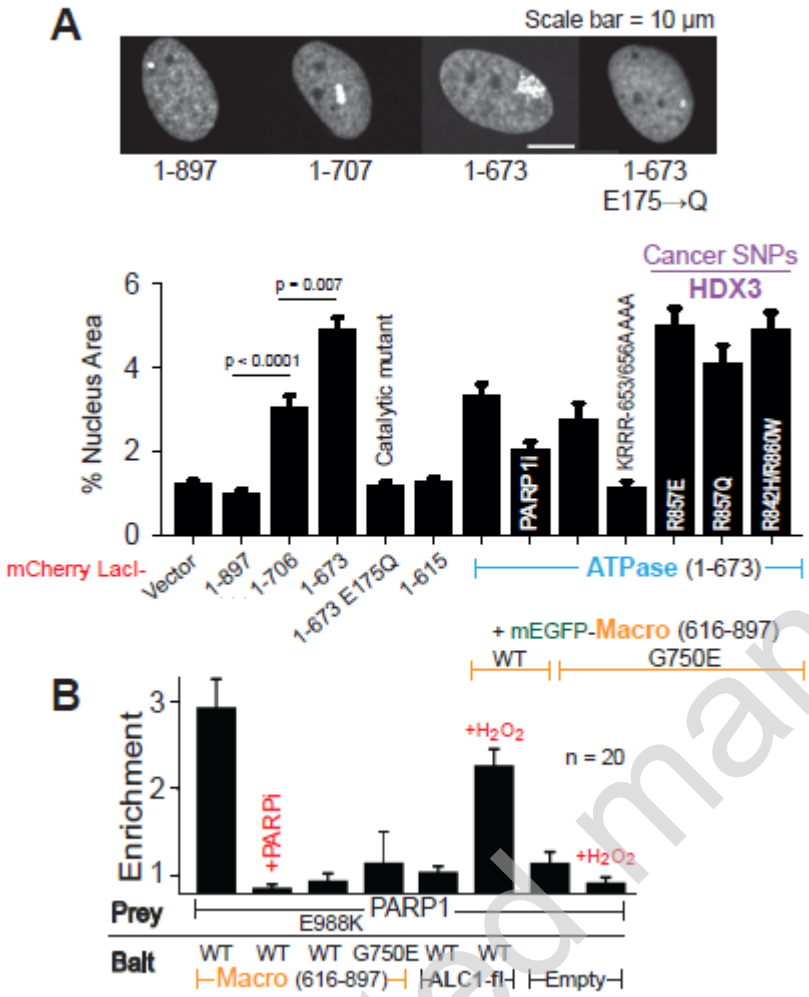

Figure 5. Release from auto-inhibition drives ALC1's chromatin remodeling *in vivo*

Modular allostery restrains the remodeler's PARP1-induced activation(A) Tethering of engineered active mCherry-LacI-ALC1 constructs to an integrated LacO array idecompacts chromatin in human U2OS cells decompacts chromatin (representative images; *top*). The decompaction of the LacO array is calculated as percent of the nucleus area (*bottom*). The deletion of ALC1's macrodomain generates a constitutively active ALC1 fragment that decompacts the LacO-array *in vivo*. Constructs assayed: full length ALC1 (1-897), macrodomain deletion (1-707), a hyperactive construct (1-674673), including in the context of an ATPase-dead point mutation (1-673, E175Q), plus ALC1 (1-615), which represents the ATPase module

identified in our limited proteolysis (Figure S1). Importantly, co-transfection of the ALC1 macrodomain (mEGFP-616-897) with the constitutively active ALC1 fragment (1-673) reduces the decompacted area. Further, cancer SNPs within HDX3 that disrupt interaction with the ATPase module (Figure 4C) do not decrease the chromatin decompaction catalyzed by the ALC1 ATPase (1-673) module.

(B) F2H assay testing the interaction of tethered ALC1 macrodomain (wild-type, WT; G750E mutant) with fluorescently tagged PARP1 (wild-type and E988K PAR elongation mutant). Indicated experiments were done in the presence of a PARP1 chemical inhibitor (+PARPi) or H_2O_2.

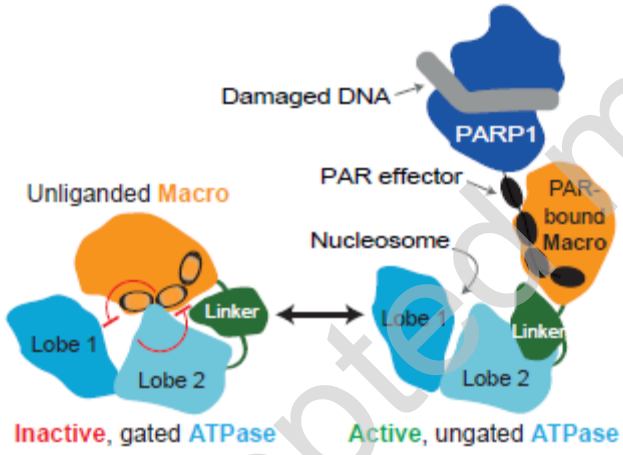

Figure 6. Modular allostery sets a threshold for PARP1-induced ALC1 activation

(C) Modular allostery in the chromatin remodeler ALC1 regulates auto-inhibition through the reciprocal interaction of ALC1's ATPase and macrodomain modules. This helps to ensure that the PARP1 product PAR acts as an allosteric activator and molecular trigger of ALC1-promoted chromatin relaxation only once PARP1 activity has been induced by acute DNA damage.

LEGENDS FOR SUPPLEMENTAL MOVIES

Movie S1

The oncogene ALC1 promotes the rapid decompaction of human chromatin structure upon laser-induced DNA damage activation. The sample movie was taken from the quantitative analysis already reported in our previous collaborative publication (7). Imaging was conducted with U2OS cells expressing a photo-activatable histone H2B protein (H2B-PATag RFP). The movie shows a time course before and after DNA damage induction of U2OS cells with endogenous levels of ALC1 enzyme (*left*; only expressing GFP) and with U2OS cells that overexpress wild-type ALC1 (*right*; expressing GFP-ALC1).

Movie S2

Acute DNA damage and PARP1 activation by UV-laser induced DNA damage triggers the release of the ALC1 ATPase module from an ALC1 macrodomain tethered to a LacO array. The movie shows the loss of interaction of the ALC1 ATPase (top movie) co-localized with the tethered macrodomain (dot at the top of each of the two nuclei in the top movie) upon laser-induced DNA damage. Notice the change of localization of the ATPase domain upon UV-laser induced DNA damage induction and PARP1 activation, as shown in the still images of Figure 2A.

Movie S3

Gain of interaction between full- length ALC1 and full length PARP1 upon DNA damage induction. Initially, prior to $H_2O_2$2-triggered DNA damage induction and PARP1 activation, PARP1 interacts very mildly with full length ALC1 tethered to the LacO array. Upon DNA damage induced, PARylation promotes the interaction, as seen by the increased localization of PARP1 on the LacO array (dot in the bottom left, which appears over time).